Protein Adhesion to Various Monomer Blends

Matthew Rusin, Ying Mei, Delphine Dean.
Department of Bioengineering, Clemson University, Clemson, SC 29634

Statement of Purpose: Finding appropriate materials for medical devices, especially implantable devices, is an issue that has plagued doctors for years. Recently [1], automated microfabricated polymer arrays were created to quickly assay multiple monomer combinations for suitability with human embryonic stem (hES) cell colony formation. This study aims to determine the fundamental protein-polymer surface interactions that could help predict the observed cell affinity results. The goal of this study is to determine whether surfaces with high affinity to vitronectin correlate with surfaces that have high colony formation.

Methods: In order to test this, a microfabricated array of 48 polymers was created [1]. The polymers were systematic combinations of pairs of monomers [1]. Each pair consisted of one major monomer and one minor monomer [1]. The major monomer was 1 of 16 monomers and made up >50% of the pair. The minor monomer was 1 of 6 monomers and made up <50% of the pair. Each polymer was deposited as a nanoliter dot on a standard glass slide that was pre-coated with poly(2-hydroxyethyl methacrylate), and polymerized [2]. In order to determine the adhesion properties of vitronectin to each polymer, force measurements were performed using Veeco Dimension 3100 atomic force microscopy (AFM) with a vitronectin-coated tip. The cantilevers were functionalized by emersion in 11-amino-1-undecanethiol for 5 minutes then rinsed with PBS. 5µL of vitronectin was then added drop-wise to the cantilevers, then submerged in PBS and allowed to bind for 2 hours. Force measurements were done in PBS at room temperature. Each polymer dot is replicated 12 times on the slide array. 5 force measurements were taken per dot, for a total of 60 data points per polymer [1].

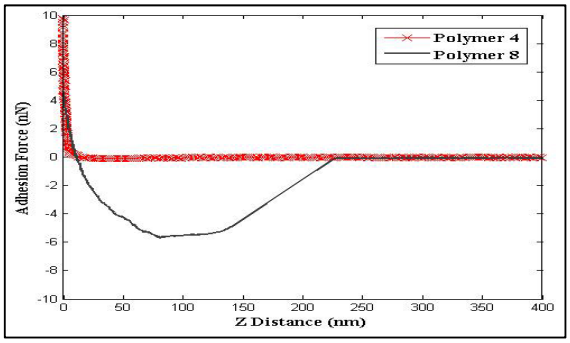

Figure 1: Comparison of 2 polymers adhesion forces.

Results: Vitronectin adhesion to the polymer surface is characterized by the retraction curve from AFM (Fig.1). Vitronectin had very little adhesion to some of the polymers (e.g., polymer 4). However, large long-range adhesions were observed on some of the polymers (e.g., polymer 8) indicating that vitronectin bound and was stretched out during the pulling process. Some polymer dots showed large variations in adhesion values between each sample and location (Figure 2).

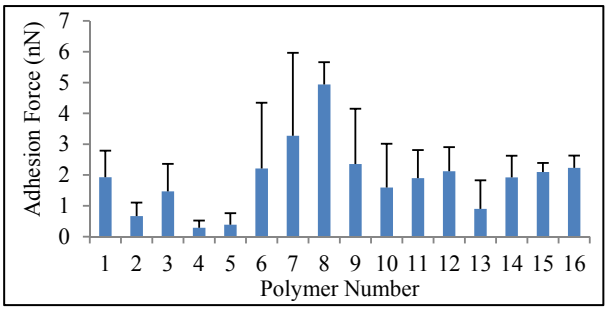

Figure 2: Vitronectin adhesion measured on 16 different polymer dots taken from one microfabricated array.

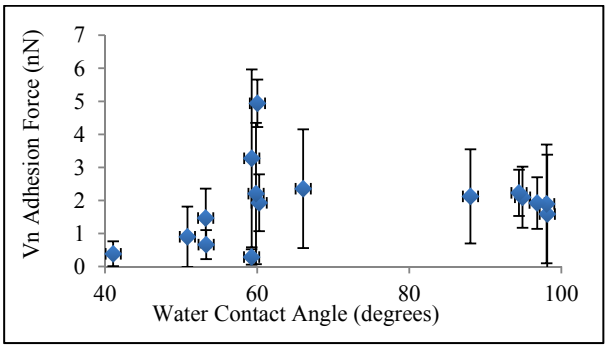

Figure 3: Comparison of average vitronectin adhesion force to the contact angle measured on each polymer dot.

It is also important to understand the wettability of each polymer and how that affects protein adhesion. In Figure 3, the adhesion force is plotted against the water contact angle. The plot indicated a higher adhesion force can be found at a moderate water contact angle (~ 60^{O}). This is consistent with our previous results that materials with moderate water contact angle tend to promote hES cell colony formation.

Conclusions: Based upon these results, it can be seen that certain polymers have high adhesion traits with vitronectin, while others have practically none. Notably,, high Vn adhesion force can be found on materials with moderate water contact angles. The large standard deviations observed on some polymers may be because in these polymers the monomers did not combine evenly leaving pockets of the minor monomer that would significantly impact measurements if the AFM probe was placed on one of those locations. Finally, comparing the results here to previously published data on stem cell colony formation [1] suggests that surfaces that show high (>3nN) adhesion forces with vitronectin tend to have larger colony formation.

References: [1] Mei, Y. Nat materials. 2010; 9: 768-778. [2] Anderson DG. Nat Biotech. 2004; 22: 863-866.